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Tumor necrosis factor-α and acute-phase proteins in early pregnant ewes after challenge with peptidoglycan-polysaccharide

T.L. Dow[a], G. Rogers-Nieman[a], I. Holásková[a], T.H. Elsasser[b], R.A. Dailey[a,*]

[a] Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV

[b] Bovine Functional Genomics Laboratory, Agricultural Research Service, USDA, Beltsville, MD

Abstract

Bacterial infection shortly after mating interferes with establishment of pregnancy. Injection of peptidoglycan-polysaccharide (PG-PS), a component of gram-positive bacteria, into sheep on day 5 after mating reduces pregnancy rate. Experiments were designed to evaluate the acute-phase response (APR) in ewes to injection of PG-PS on day 5 after mating (day 0). Catheters were inserted into the jugular and posterior vena cava on day 4. On day 5, ewes were challenged with saline or 30 μg/kg body weight (BW) PG-PS (Exp 1) or 60 μg/kg BW PG-PS (Exp 2). Blood samples were collected every 15 min for 6 h (Exp 1) and every 15 min for 2 h, hourly for 12 h, and at 24, 36, and 48 h (Exp 2). Body temperature and clinical signs of infection were monitored in Exp 2. Plasma was assayed for concentrations of a pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α); 2 APR proteins, serum amyloid A (SAA) and haptoglobin (Hp); and progesterone (P_4). Ewes injected with 60 μg/kg BW PG-PS exhibited fever, vaginal discharge, loss of appetite, and lethargy. After challenge with either 30 μg/kg or 60 μg/kg BW PG-PS, TNF-α increased in the posterior vena cava. Concentrations of SAA and Hp in the jugular increased after challenge with 60 μg/kg BW PG-PS. Only half (5/10) of the ewes treated with 60 μg/kg BW PG-PS had ultrasonically visible embryos, and none of them had functional corpora lutea (CL) (<1 ng/mL of P_4) on day 21. On the other hand, 8/9 (88.9%) control ewes had visible embryos and all had functional CL on day 21. Using logistic regression, pregnancy on day 21 was predicted to depend on concentrations of TNF-α and Hp on day 5 and concentration of P_4 on day 14. In summary, injection of PG-PS on day 5 after mating resulted in fever; increased concentrations of TNF-α, Hp, and SAA on the day of and the day after the PG-PS challenge; and decreased concentrations of P_4 on days 14 and 21. These factors were related to failure to establish pregnancy.

Keywords: Pregnancy; Sheep; Peptidoglycan-polysaccharide; gram-positive bacteria; Haptoglobin; SAA; TNF-α

1. Introduction

Occurrence of mastitis during early embryonic development interferes with establishment of pregnancy and extends days open in dairy cattle [1]. Because immunization to peptidoglycan-polysaccharide (PG-PS) or whole killed *Streptococcus pyogenes*, gram-positive bacteria, did not improve establishment of pregnancy after a challenge on day 5 after mating (day 0) in ewe lambs, even though IgG anti-PG-PS titers were high [2], the innate immune system might have a more important role than the humoral. Bacteria stimulate macrophages and monocytes [3] to release pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), leading to the acute-phase response (APR). The APR is defined by secretion of fibrinogen,

T. L. Dow and G. Rogers-Nieman contributed an equal amount of work and are both considered first author.

* Address for correspondence: Division of Animal and Nutritional Sciences, PO Box 6108, West Virginia University, Morgantown, WV 26506-6108, USA; Phone: (304) 293-2631; Fax: (304) 293-2232.

E-mail address: rdailey@wvu.edu (R.A. Dailey).

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serum amyloid A (SAA), haptoglobin (Hp), α-1 acid glycoprotein, and C-reactive protein, principally by the liver [4]. Increased production of TNF-α, induced by injection of lipopolysaccharide (LPS), a component of the cellular wall of gram-negative bacteria, was associated with failure to establish pregnancy [5]. Injection of peptidoglycan-polysaccharide (PG-PS), a component of the cell wall of gram-positive bacteria, on day 5 to naturally mated ewes reduced conception rates [6].

The primary objective of this study was to validate induction of an innate immune response after challenging day 5 pregnant ewes with PG-PS. Changes in concentrations of TNF-α, a pivotal pro-inflammatory cytokine, in venous blood draining the reproductive tract and in the systemic circulation, and in systemic profiles for 2 APR proteins, SAA and Hp, were measured and associated with establishment of early pregnancy.

2. Materials and methods

2.1. Animals

Suffolk ewes (~80 kg body weight [BW]) were used under an approved institutional animal care protocol (WVU IACUC #9801-05). Ewes were fed corn silage and haylage twice daily and had ad libitum access to fresh water. Estrus was synchronized [7] in 2 consecutive breeding seasons (Exp 1 and Exp 2) and detected by vasectomized rams with marking harnesses (one ram/12 ewes). On the third day after ram introduction, the unmarked ewes (expected to be $\geq$ day 4 of the estrous cycle) were injected with prostaglandin F2α (PGF2α) i.m., 2 $\times$ 5 mg, 3 h apart (Lutalyse, Upjohn-Pharmacia, Kalamazoo, MI). After the second injection of PGF2α, intact rams with marking harnesses were placed with these ewes for mating. This mating scheme routinely achieves 60%-80% conception rates [7].

In Exp 1 (n = 10) and in Exp 2 (n = 20), ewes that were mated at least twice (first mating = day 0 of gestation) were assigned at random to 1 of 2 groups, control or PG-PS (100P PG-PS, BD Lee Laboratories, Grayson, GA)]. On day 4, ewes in Exp 1 were sedated with diazepam (0.3 mg/kg BW i.v.; Hoffmann-La Rouche Inc., Nutley, NJ) and those in Exp 2 were sedated with diazepam and ketamine HCl (0.7 mg/kg BW; Phoenix Pharmaceuticals, Inc., Mountain View, CA); ewes were then anesthetized using isoflurane or halothane gas via an intratracheal tube. The right hock was shaved, scrubbed with Betadine (The Perdue-Frederick Co., Stamford, CT), and treated locally with lidocaine (Butler Company, Columbus, OH). The skin was tented using forceps and slit longitudinally approximately 1 cm using sterile surgical blades (Propper Manufacturing Co., Inc., Long Island City, NY). The saphenous vein, located about 3 cm lateral to the Achilles tendon, was isolated by blunt dissection and incised. A polyvinyl catheter (id 0.040″ and od 0.070″) was passed into the saphenous vein for 50 (Exp 1) or 55 cm (Exp 2) to sample the posterior vena cava draining the reproductive tract [8]. Catheters were flushed with 2 mL of heparinized saline (250 U heparin [Sigma, Atlanta, GA]/mL of 0.9% NaCl) and capped with a removable plug. The catheter site was closed with suture, dressed with Furazolidone (Veterinary Products Laboratories, Phoenix, AZ), covered with sterile gauze, and wrapped with 3M Vetrap bandaging tape (Minnesota Mining Mfg, St. Paul, MN). A venous catheter (18-gauge, 8-inch; I-CATH; Chartermed, Inc., Lakewood, NJ) was placed in the right jugular vein. The area was treated topically with Furazolidone, covered with gauze, and wrapped with Vetrap.

2.2. Treatment groups

Ewes were housed in groups of 5 ewes per outdoor barn pen (~7 °C, Exp 1) or 2 ewes per pen in an enclosed facility (~20 °C, Exp 2). On day 5 after mating, ewes were injected (left jugular) with 30 μg/kg BW PG-PS (n = 6, 30 μg/kg for 80-kg sheep is equivalent to 2.4 mg of rhamnose/ewe) or 0.9% NaCl (control, n = 4) in Exp 1 or with 60 μg/kg PG-PS (n = 10, 60 μg/kg for 80-kg sheep is equivalent to 4.8 mg of rhamnose/ewe) or control (n = 10) in Exp 2. When compared to saline injection, these doses resulted in fewer pregnancies when ewes were injected on day 5 [6]. In Exp 1, blood samples were collected from each catheter prior to and every 15 min for 6 h after treatment into heparinized, chilled tubes and kept on ice until centrifuged. In Exp 2, samples were collected into EDTA-treated tubes prior to and every 15 min for 120 min, every hour for 12 h, and every 12 h until 48 h. Aliquots of plasma were stored at −20 °C. On days 14 and 21 of Exp 2, jugular samples were collected into EDTA-treated tubes for measuring concentrations of P_4; animals with concentrations above 1 ng/mL were deemed to be pregnant [9]. In addition, transrectal ultrasonography on day 21 was used to visualize pregnancy using a 7.5-MHz transducer (Corometrics Medical Systems, Inc., Wallingford, CT) and an Aloka 500 ultrasound console (Corometrics) [10]. One control ewe from Exp 2 was diagnosed with pneumonia on day 21 and was excluded from the experiment. Body temperatures were monitored with a rectal thermometer, and behavior and/or physical appearances were recorded every 12 h.

2.3. Plasma analysis

Concentrations of TNF-α in duplicate plasma samples (100 μL) were determined by radioimmunoassay [11] with intra-assay CV $<$10% for Exp 1 and $<$7% for Exp 2. The minimal detectable quantity of recombinant bovine TNF-α (Ciba Geigy GmbH, St. Aubin, Switzerland) corresponded to 7 pg/tube, whereas typical recovery of nonlabeled TNF-α added to bovine plasma averaged 97.4%. A multi-species SAA solid-phase enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Inc., Camarillo, CA) was used in Exp 2 on samples that were diluted 1:3000. The assay had a sensitivity of 0.3 μg/mL and intra-assay CV of 9.8%. Concentrations of Hp were determined using multi-species Hp colorimetric kits (Phase Range, Tri-Delta Diagnostics, Inc., Morris Plains, NJ) in duplicate samples diluted 1:3 with sensitivity of 0.05 mg/mL and intra-assay CV of 6.9%. All ELISA plates were analyzed using SPECTRAmax PLUS^{384} and Softmax PRO software, version 4.6 (Molecular Devices Corporation, Sunnyvale, CA, 2003) at an optical density of 450 nm. Concentrations of P_4 on days 14 and 21 were assayed using a validated radioimmunoassay [12] with assay sensitivity of 0.20 ng/mL and intra-assay and inter-assay CV of 7% and 14.8%, respectively.

2.4. Statistical analysis

Continuous data for body temperature and for concentrations of TNF-α, SAA, and Hp were analyzed using the repeated-measures analysis of variance (ANOVA) of the PROC MIXED procedure of SAS, version 9.1.3 (SAS Institute, Inc., Cary, NC, 2004). Concentrations of P_4 were analyzed using ANOVA and multiple-comparison tests. The categorical data, such as the incidence of lethargy, vaginal discharge, loss of appetite, and pregnancy rate, were analyzed with the Fisher exact test using the Contingency Analysis platform of JMP version 6.0 (SAS, 2005). Logistic regression (PROC GENMOD, link = logit; SAS) was used to predict the success of pregnancy based on the concentrations of SAA, Hp, and TNF-α on days 5-6, and P_4 on days 14 and 21. Significance was determined for P values less than 0.05.

3. Results

3.1. TNF-α

The pro-inflammatory cytokine TNF-α was measured in samples from both the vena cava and the jugular vein. In Exp 1, 30 μg/kg BW PG-PS increased (P = 0.002) concentrations of TNF-α in the vena cava within the first 2 hours after injection (Fig. 1A). Concentrations of TNF-α in the jugular vein did not differ ($P > 0.05$) between treated and control ewes (Fig. 1B). Likewise, in Exp 2, treatment with 60 μg/kg BW PG-PS resulted in higher concentrations of TNF-α in the vena cava (P = 0.003) than in the jugular vein (Fig. 1C). A sustained (P = 0.006) increase, expressed as the percent change from hour 0 (Fig. 1D), in concentrations of TNF-α in samples from the jugular vein in PG-PS-treated ewes lasted up to 12 h after PG-PS injection but was similar to control values at 24 h.

3.2. Acute-phase proteins

Concentrations of SAA for ewes treated with 60 μg/kg BW PG-PS differed ($P < 0.001$) from those in controls, with up to a 5-fold increase compared to baseline values (Fig. 2A). The increased concentration of SAA in treated ewes persisted through 24 h after PG-PS injection. Concentrations of Hp in ewes began to increase at 1 h after treatment with 60 μg/kg BW PG-PS and continued to increase through 24 h (Fig. 2B). An effect of time (P = 0.003) was detected in addition to an effect of treatment ($P < 0.001$), with concentrations increasing in PG-PS ewes but not control ewes.

3.3. Fever and clinical signs of inflammation

Ewes treated with PG-PS (n = 10) had their highest body temperature at 12 h after treatment, whereas body temperatures in control ewes (n = 10) remained unchanged (Fig. 3). Body temperature was still elevated in PG-PS-treated ewes at 24 h when compared to control ewes (treatment-by-time interaction, $P < 0.001$). Beginning about 3 hours after the injection of PG-PS, challenged ewes had a muco-purulant vaginal discharge, which persisted for 24 h and cleared to a watery discharge by 48 h. The discharge was observed in all treated ewes but was not observed in control ewes ($P <$ 0.001). Lethargy and loss of appetite were not observed in control ewes, but lethargy was observed in 4 PG-PS-treated ewes (P = 0.05), and loss of appetite was observed in 3 PG-PS-treated ewes (P = 0.12). None of the ewes experienced changes in incidences of coughing, nasal discharge, or watery eyes.

3.4. Progesterone and pregnancy diagnosis

Mean P_4 was lower ($P < 0.005$) in PG-PS-treated (1.35 $\pm$ 0.34 ng/mL) compared to control (2.36 $\pm$ 0.18 ng/mL) ewes on day 14. Although statistically lower, all treated ewes had physiological concentrations of P_4

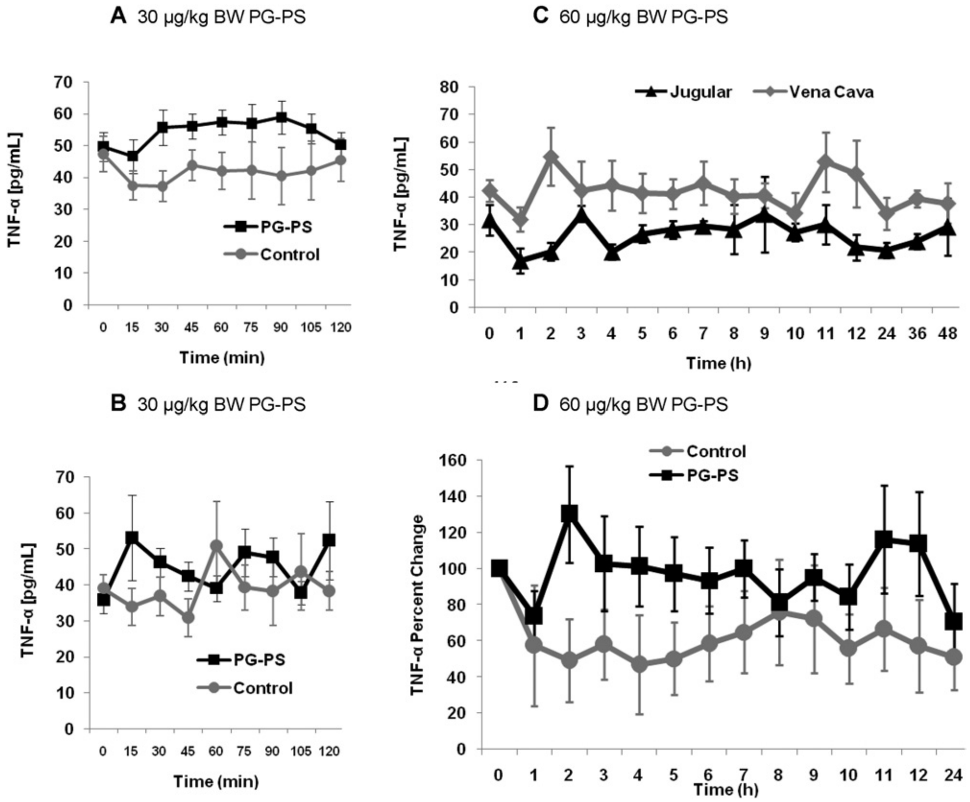

Fig. 1. Concentrations of tumor necrosis factor-α (TNF-α) in vena cava and jugular vein. Panel A shows increased ($P = 0.002$) concentrations of TNF-α in the vena cava after injection of 30 μg/kg body weight peptidoglycan-polysaccharide (PG-PS) but not ($P > 0.05$) the jugular vein (Panel B). Treatment with 60 μg/kg body weight PG-PS resulted in higher concentrations of TNF-α in vena cava ($P = 0.003$) then in the jugular vein (Panel C). Panel D shows effect ($P = 0.006$) of PG-PS on TNF-α in jugular measured as percentage of hour 0.

indicative of an active CL (Fig. 4A). However, on day 21, concentrations of P_4 in the ewes treated with 60 μg/kg BW PG-PS were indicative of regressing or regressed CL (0.63 $\pm$ 0.13 ng/mL) as compared to the maintained CL in controls (2.33 $\pm$ 0.17 ng/mL, Fig. 4A, $P < 0.001$). Very low P_4 concentrations in all

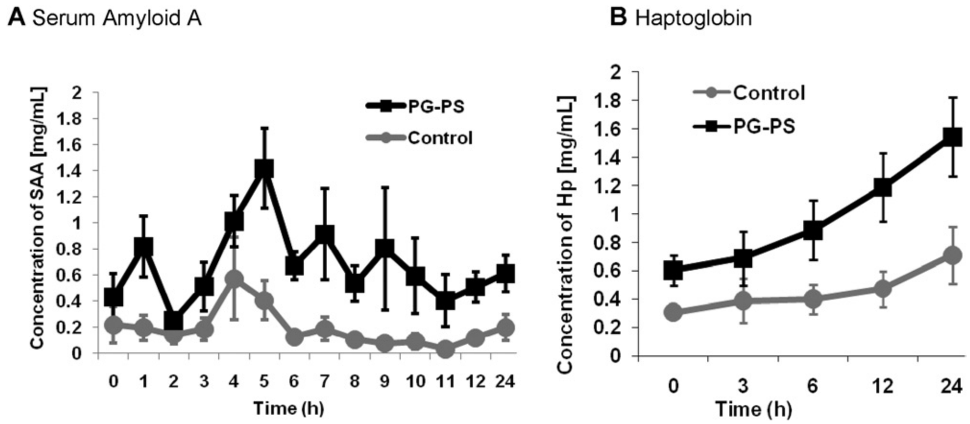

Fig. 2. Serum concentrations of acute-phase proteins. Treatment with 60 μg/kg body weight peptidoglycan-polysaccharide increased ($P = 0.001$) concentrations of serum amyloid A (SAA, Panel A) and haptoglobin (Hp, $P = 0.003$, Panel B).

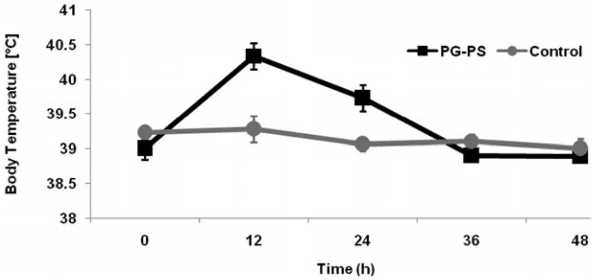

Fig. 3. Body temperature. Body temperature in peptidoglycan-polysaccharide-treated ewes (n = 10) increased compared to control (n = 10) ewes (treatment, $P = 0.016$; time, $P < 0.001$; and interaction of treatment and time, $P < 0.001$).

PG-PS-treated ewes (≤1 ng/mL) on day 21, implied a zero pregnancy rate (Fig. 4B). In contrast, all control ewes had sustained or showed increased P_4 (range 1.4 to 3.3 ng/mL) on day 21 (Fig. 4B, $P < 0.05$), indicating a 100% pregnancy rate. Evidence of embryonic tissue or embryonic membranes was detected on day 21 in 50% (5/10) of the treated group and 88.9% in controls (8/9 ewes, Fig. 4B; $P = 0.09$). At this early stage, viability of embryos measured by ultrasonic heart beat was not observable.

3.5. Prediction model

Logistic regression indicated a relationship between concentrations of Hp ($P = 0.003$) and TNF-α ($P = 0.016$) on day 5 and of P_4 on day 14 ($P = 0.004$) and success of pregnancy on day 21.

4. Discussion

Immune and endocrine systems interact multiple times to establish pregnancy in mammals. For instance, an inflammatory response to semen occurs on the day of mating and is characterized by massive inflammatory leukocyte infiltration and heightened cytokine gene expression in the uterus [13]. Ironically, after opsonizing the bacterial pathogens after mating, the immune system switches to downplay its defense mechanisms for the duration of early pregnancy until maternal recognition, implantation, and establishment of pregnancy. However, unexpected inflammatory insults during early pregnancy, for example acute mastitis, can compromise reproductive success in cows and sheep [1,6,14–16]. To mimic inflammatory response to mastitis in early pregnant cows, PG-PS was injected in early pregnant sheep (day 5 after mating), and immune factors were examined for their potential to interfere with establishment of pregnancy. A systemic immune response was evident, as treated ewes showed fever, associated behavioral changes, vaginal discharge, and increased concentrations of TNF-α, SAA, and Hp on the day of the challenge.

Serum amyloid A is used as a biomarker of infection, inflammation, and trauma in many species, including ruminants [17,18]. Increased circulating concentrations of Hp were measured in ewes that had intrauterine infection [19]. Challenge with endotoxin (LPS), a component of gram-negative bacteria, stimulated liver macrophages to secrete TNFα, interleukin (IL) IL-1, and

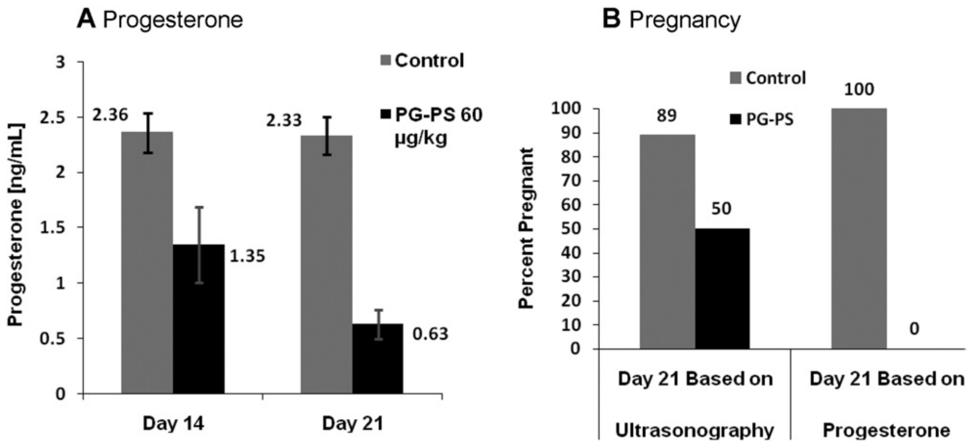

Fig. 4. Pregnancy determination. Concentrations of plasma progesterone (P_4) on days 14 and 21 after mating (Panel A). One control ewe was excluded because on day 21 she was diagnosed with pulmonary infection. Peptidoglycan-polysaccharide (PG-PS) decreased P_4 concentration on both days ($P = 0.005$ and $P < 0.001$, respectively). By ultrasonographic diagnosis of embryonic tissues or embryonic membranes on day 21 (Panel B), 50% of PG-PS ewes and 88.9% of control ewes were pregnant ($P = 0.09$). Based on P_4 concentration on day 21, all control ewes but no PG-PS ewes were pregnant.

IL-6, which then induced hepatocytes to synthesize and secrete SAA and Hp [20]. Although PG-PS binds to different toll-like receptors (TLR 2 and TLR 6) [21] on macrophages than LPS (TLR 4) [22], the PG-PS binding leads to activation of nuclear factor κB (NF-κB) and, subsequently, secretion of inflammatory cytokines [23]. Ewes treated with PG-PS had sustained, increased concentrations of TNF-α, and 5-fold and 3-fold increases in SAA and Hp within 1 and 6 h, respectively. Haptoglobin has bacteriostatic properties and antagonizes TNF-α to decrease inflammation [24], thus creating a negative feedback loop. In vitro, Hp, one of many proteins secreted by the oviductal epithelium that form a protective protein coat around the blastocyst in the rabbit [25], antagonized host immune response to LPS by suppressing production of TNF-α in monocytes. Possibly, a first step in establishing early pregnancy after the bacterial challenge is masking the allogenic embryo with maternal proteins [26], in particular, enhanced secretion of Hp, in an attempt to protect the embryo from uterine macrophages and mast cells.

Results of the present experiment show increasing concentrations of SAA (marker of inflammation), TNF-α (pro-inflammatory cytokine), as well as Hp (anti-inflammatory agent) after PG-PS challenge. Infection following mastitis, which leads to increases in SAA and Hp [27,28], interferes with establishment of pregnancy in dairy cattle [1]. Failure to continue pregnancy after bacterial challenge could be through (a) direct effects of the maternal inflammatory mediators on the embryo or (b) an indirect effect via the endocrine system. Maternal, but not fetal, factors [29] resulted in embryonic death in an LPS-hyporesponsive strain of mice. Challenges with LPS activated macrophages and natural killer (NK) cells to synergistically produce large amounts of TNF-α local to the embryo [5,30–32]. In vitro, addition of TNF-α to the cultures of mouse [33] and cow [34] blastocysts inhibited their development. In the present study, production of TNF-α in the reproductive tract, presumably reflected by higher concentrations in the posterior vena cava compared to the jugular vein after PG-PS treatment, could have interfered with the establishment of pregnancy by acting through toll-like receptors to directly promote embryonic death [35].

Alternatively, modified maternal conditions, such as endocrine or metabolic functions, could affect establishment of pregnancy. Specifically, bacterial-induced immune challenge could interfere with the function of the CL or result in failure of maternal recognition of pregnancy, which also eventually leads to compromised CL function. Trophoblastic interferon (IFN-τ) acts as the anti-luteolysin and maternal recognition of pregnancy factor in ruminants and has antiviral, anti-proliferative, and immunomodulatory effects [36,37]. The mRNA for IFN-τ is detectable as the blastocyst hatches from the zona pellucida [38] and reaches its peak in the ewe on day 13.

Progesterone plays a large role in the "switch" of the immune system in the uterus [39,40]; progesterone has been described as an immuno-inhibitory hormone because it down-regulates pro-inflammatory immune activities. Progesterone administered twice daily to both ovariectomized and intact postpartum ewes before intrauterine inoculation with bacteria suppressed uterine immunity [41]. In Exp 2 of the present study, when compared to controls, concentration of P_4 shortly after the time of maternal recognition of pregnancy (day 14) was lower in PG-PS-treated ewes. Although the concentration of P_4 was physiologically sufficient (>1 ng/mL) to maintain pregnancy, luteal function was obviously compromised. Furthermore, using logistic regression, the concentration of P_4 on day 14 was a significant predictor of pregnancy success. On day 21, concentration of P_4 was even lower (< 1 ng/mL) in PG-PS-treated ewes, indicating that the CLs were regressing, if they were not already regressed. Therefore, the gradually decreasing CL function was a result of unsuccesful maternal recognition of pregnancy and not the cause of the embryonic death. A secondary indicator of pregnancy success was ultrasonic examination on day 21. Although the proportion of pregnant ewes in the PG-PS group (50%) was not statistically different compared to controls (88.9%), sample size was small for categorical analysis of pregnancy diagnosis, reflected in a low power of the statistical test, yet the difference in pregnancy rates may be quite significant financially, for example for the dairy cow producer. The discrepancy between results for pregnancy diagnosis by concentrations of P_4 compared to ultrasonic observations may be explained by gradual embryonic demise, such as some embryos carried in ewes with P_4 less than 1 ng/mL might be ultrasonically visible, but not viable.

The combination of increased SAA, Hp, and TNF-α on day 5-6 and decreased P_4 on day 14 were significant predictors of pregnancy success on day 21. Hence, both direct and indirect effects on pregnancy success likely were induced. Specifically, elevated local TNF-α might induce apoptosis of trophoblastic cells, thereby causing failure to produce adequate concentrations of IFN-τ necessary to maintain the CL. This failure would lead to decreased P_4 and ultimately, failure to establish pregnancy.

5. Conclusions

Injection of PG-PS into the jugular vein induced an immune reaction as shown by fever, vaginal discharge, and increases in SAA, TNF-α, and Hp on the day of and the day after the challenge. These changes were followed by decreases in progesterone on days 14 and 21. Interference with establishment of early pregnancy in ewes inoculated with PG-PS appears to be from local uterine inflammatory response, as shown by elevated TNF- α in the posterior vena cava.

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References

[1] Barker AB, Schrick FN, Lewis MJ, Dowlen HH, Oliver SP. Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. J Dairy Sci. 1998;81:1285–1290.

[2] Holásková I, Lewis GS, Elliott M, Blemings KP, Dailey RA. Effect of peptidoglycan-polysaccharide complex on reproductive efficiency in sheep. Am J Reprod Immunol. 2004;52:197–203.

[3] Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology, the Immune System in Health and Disease, 6th ed. New York, NY: Garland Science Publishing; 2005.

[4] Vels L, Røntved CM, Bjerring M, Ingvartsen KL. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. J Dairy Sci. 2009;92:922–934.

[5] Gendron RL, Nestel FP, Lapp WS, Baines MG. Lipopolysaccharide-induced fetal resorption in mice is associated with the intrauterine production of tumor necrosis factor-alpha. J Reprod Fertil. 1990;90:395–402.

[6] Stewart A, Inskeep EK, Townsend EC, Dailey RA. Effects of Gram-positive bacterial pathogens in ewes: peptidoglycan as a potential mediator of interuption of early pregnancy. Reproduction. 2003;125:295–299.

[7] Inskeep K, Lewis P, Stilley N, Mulledy R, Dinsmore H. Synchronization of Estrus as a Management Tool in the Ewe Flock. Morgantown, WV: Agricultural and Forestry Experiment Station, West Virginia University; 1983.

[8] Benoit AM, Dailey RA. Catheterization of the caudal vena cava via the lateral saphenous vein in the ewe, cow, and gilt: an alternative to utero-ovarian and medial coccygeal vein catheters. J Anim Sci. 1991;69:2971–2979.

[9] Susmel P, Piasentier E. Assessment of pregnancy in Bergamsca ewes by analysis of plasma progesterone. Small Rumin Res. 1992:8:325–332.

[10] Shrick FN, Inskeep EK. Determination of early pregnancy in ewes utilizing transrectal ultrasonography. Theriogenology. 1993;40:295–296.

[11] Kenison DC, Elsasser TH, Fayer R. Radioimmunoassay for bovine tumor necrosis factor: concentrations and circulating molecular forms in bovine plasma. J Immunoassay. 1990;11:177–198.

[12] Sheffel CE, Pratt BR, Ferrell WL, Inskeep EK. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J Anim Sci. 1982;54:830–836.

[13] Dalton T, Kover K, Dey SK, Andrews GK. Analysis of the expression of growth factor, interleukin-1, and lactoferrin genes and the distribution of inflammatory leukocytes in the preimplantation mouse oviduct. Biol Reprod. 1994;51:597–606.

[14] Schrick FN, Hockett ME, Saxton AM, Lewis MJ, Dowlen HH, Oliver SP. Influence of subclinical mastitis during early lactation on reproductive parameters. J Dairy Sci. 2001;84:1407–1412.

[15] Santos JEP, Cerri RLA, Ballou MA, Higginbotham GE, Kirk JH. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim Repro Sci. 2004;80:31–45.

[16] Chebel RC, Santos JEP, Reynolds JP, Cerri RLA, Juchem SO, Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci. 2004;84:239–255.

[17] Eckersall PD, Lawson FP, Bence L, Waterston MM, Lang TL, Donachie W, Fontaine MC. Acute phase protein response in an experimental model of ovine caseous lymphadenitis. BMC Vet Res. 2007;19:3–35.

[18] Molenaar AJ, Harris DP, Rajan GH, Pearson ML, Callaghan MR, Sommer L, Farr VC, Oden KE, Miles MC, Petrova RS, Good LL, Singh K, McLaren RD, Prosser CG, Kim KS, Wieliczko RJ, Dines MH, Johannessen KM, Grigor MR, Davis SR, StelwagenK. The acute-phase protein serum amyloid A3 is expressed in the bovine mammary gland and plays a role in host defence. Biomarkers. 2009;14:26–37.

[19] Regassa F, Noakes DE. Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. Vet Rec. 2002;150:605–607.

[20] Jacobsen S, Andersen PH, Toelboell T, Heegaard PMH. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. J Dairy Sci. 2004;87:3330–3339.

[21] Pasare C, Medzhitov R. Toll-like receptors and acquired immunity. Semin Immunol. 2004;16:23–26.

[22] Dziarski R, Gupta D. Role of MD-2 in TLR-2 and TLR4-mediated recognition of Gram-negative and Gram-positive bacteria and activation of chemokine genes. J Endotoxin Res. 2000;6:401–405.

[23] Takeda K, Akira S. TLR signaling pathways. Semin Immunol. 2004;16:3–9.

[24] Arredouani MS, Kasran A, Vanoirbeek JA, Berger FG, Baumann H, Ceuppens JL. Haptoglobin dampens endotoxin-in-

duced inflammatory effects both in vitro and in vivo. Immunology. 2005;114:263–271.

[25] Herrler A, Krusche CA, Müller-Schöttle F, Beier HM. Haptoglobin expression and release by rabbit oviduct and endometrium, its localization in blastocyst extra-embryonic matrix and fluid during preimplantation time. Hum Reprod. 2004;19:2730–2737.

[26] Lavery K, Way A, Killian G. Identification and immunohistochemical localization of a haptoglobin-like protein in the tissues and fluids of the bovine (Bos taurus) ovary and oviduct. Reproduction. 2003;125:837–846.

[27] Grönlund U, Hallén Sandgren C, Persson Waller K. Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. Vet Res. 2005;36:191–198.

[28] Suojala L, Orro T, Järvinen H, Saatsi J, Pyörälä S. Acute phase response in two consecutive experimentally induced E. coli intramammary infections in dairy cows. Acta Vet Scand. 2008;50:18.

[29] Silver RM, Lohner SW, Daynes RA, Mitchell MD, Branch DW. Lipopolysaccharide-induced fetal death:The role of tumor-necrosis factor alpha. Biol Reprod. 1994;50:1108–1112.

[30] Chaouat G, Menu E, Clark DA, Dy M, Minkowski M, Wegmann TG. Control of fetal survival in CBA X DBA/2 mice by lymphokine therapy. J Reprod Fertil. 1990;80:447–458.

[31] Duclos AJ, Haddad EK, Baines MG. Presence of activated macrophages in a murine model of early embryo loss. Am J Reprod Immunol. 1995;33:354–366.

[32] Baines MG, Duclos AJ, Antecka E, Haddad EK. Decidual infiltration and activation of macrophages leads to early embryo loss. Am J Reprod Immunol. 1997;37:471–477.

[33] Baines MG, Duclos AJ, DeFougerolles AR, Gendron RL. Immunological prevention of spontaneous early embryo resorption is mediated by non-specific immunostimulation. Am J Reprod Immunol. 1996;35:34–42.

[34] Soto P, Natzke RP, Hansen PJ. Actions of tumor necrosis factor-alpha on oocyte maturation and embryonic development in cattle. Am J Reprod Immunol. 2003;50:380–388.

[35] Abrahams VM, Aldo PB, Murphy SP, Visintin I, Koga K, Wilson G, Romero R, Sharma S, Mor G. TLR6 modulates first trimester trophoblast responses to peptidoglycan. J Immunol. 2008;180:6035–6043.

[36] Demmers KJ, Derecka K, Flint A. Trophoblast interferon and pregnancy. Reproduction. 2001;121:41–49.

[37] Stewart HJ, McCann SHE, Northrop AJ, Lamming GE, Flint APF. Sheep antiluteolytic interferon: cDNA sequence and analysis of mRNA levels. J Mol Endocrinol. 1989;2:65–70.

[38] Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW, Roberts RM. Expression of trophoblastic interferon genes in sheep and cattle. Biol Reprod. 1990;43:210–218.

[39] Black WG, Ulberg LC, Kidder HE, Simon J, McNutt SH, Casida LE. Inflammatory response of the bovine endometrium. Am J Vet Res. 1953;14:179–183.

[40] Hawk HW, Turner GD, Sykes JF. Variation in the inflammatory response and bactericidal activity of the sheep uterus during the estrous cycle. Am J Vet Res. 1961;22:689–692.

[41] Seals RC, Wulster-Radcliffe MC, Lewis GS. Uterine response to infectious bacteria in estrous cyclic ewes. Am J Reprod Immunol. 2003;49:269–278.